UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/525,019	06/28/2005	Michael Giesing	GIES3002	4832

23364 7590 05/15/2008

BACON & THOMAS, PLLC
625 SLATERS LANE
FOURTH FLOOR
ALEXANDRIA, VA 22314

EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	**PAPER NUMBER**
1636	

MAIL DATE	DELIVERY MODE
05/15/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/525,019	GIESING ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 January 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7 and 9-17* is/are pending in the application.

4a) Of the above claim(s) *9 and 10* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7 and 11-17* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 February 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/18/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Receipt is acknowledged of an amendment, filed 2/18/2005, in which claim 8 was canceled, claims 1-7, 9 and 10 were amended, and claims 11-17 were newly added. Currently, claims 1-7 and 9-17 are pending.

Election/Restrictions

Applicant's election with traverse of Group I in the reply filed on 1/28/2008 is acknowledged. The traversal is on the ground(s) that Boer et al fails to teach the technical feature of the invention, because Boer et al fails to teach thioredoxin reductase. This is not found persuasive because Boer et al teach a 31,500-clone human cDNA array, which comprises probes to detect thioredoxin reductase, as evidenced by the GEO entry for platform GPL10, for which the general description and entries for thioredoxin reductase ([human, placenta, mRNA, 3826 nt], thioredoxin reductase: txnrd1) are provided. Accordingly, Boer et al teach the technical feature linking the invention of Groups I and II, and thus there is no special technical feature linking these inventions. The restriction between the groups is maintained.

Because new evidence is presented to support the restriction between Groups I and II, the requirement has not been made final at this time.

Claims 9-10 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 1/11/2008.

An examination on the merits of claims 1-7 and 11-17 follows.

Priority

Acknowledgment is made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d). Receipt of the certified copy of the foreign priority document, GERMANY 102 38 046.5, is acknowledged. These papers have been placed of record in the file.

Information Disclosure Statement

Receipt of an information disclosure statement, filed on 10/18/2005, is acknowledged. The signed and initialed PTO 1449 has been mailed with this action.

Specification

The disclosure is objected to because of the following informalities: at page 40, line 14 the term "RNA" is misspelled.

Appropriate correction is required.

Claim Objections

Claims 1-7 and 11-17 are objected to because of the following informalities: In claim 1, Roman numeral I is inconsistent with the numerals used to identify the second and third steps. It would be remedial to replace "I" with "i". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-7 and 11-17 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (a) obtaining a blood sample from a human subject, collecting mononuclear cells from the blood sample, removing a fraction of the mononuclear cells to obtain test fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain test fraction C;

(b) obtaining a blood samples from a healthy human subjects not suffering from cancer, collecting mononuclear cells from the blood samples, removing a fraction of the mononuclear cells to obtain reference fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain reference fraction C;

(c) isolating CD45-positive lymphocytes from reference fraction A' to obtain reference fraction A,

(d) isolating mRNA from test fraction A', test fraction C, reference fraction A, and reference fraction C;

(e) measuring the expression level of manganese superoxide dismutase (MNSOD), thioredoxin reductase (TXNRD1), and glutathione peroxidase (GPX1) in each of the mRNA samples, wherein said measuring is by reverse transcription and PCR using primers consisting of SEQ ID NOs: 1 and 2 for MNSOD, SEQ ID NOs: 4 and 5 for TXNRD1, and SEQ ID NOs: 7 and 8 for GPX1;

(f) measuring the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each of the mRNA samples;

(g) calculating for each mRNA sample the ratio of each of MNSOD, TXNRD1, and GPX1 expression to GAPDH expression;

(h) determining the average and standard deviation for the expression ratio of MNSOD, TXNRD1, and GPX1 from reference fraction C to reference fraction A for the healthy control samples, and determining a limit for expression which is the average plus one standard deviation;

(i) determining the expression ratio of MNSOD, TXNRD1, and GPX1 from test fraction C to test fraction A' of the test sample; and

(j) comparing the expression ratio for each of MNSOD, TXNRD1, and GPX1 for the test sample to the determined limit for each gene;

wherein an expression ratio higher than the limit for at least one of MNSOD, TXNRD1 or GPX1 indicates that disseminated cancer cells are present in the test blood sample, does not reasonably provide enablement for the use of any body fluid from any species of organism, the absence of a reference sample, the use of any reference sample, determining the expression of any manganese superoxide dismutase gene, any thioredoxin reductase gene, or any glutathione peroxidase gene, early diagnosis of a tumor, or estimating the risk to develop a metastasis or recurrence. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of

experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The claims are drawn to a method for investigating a body fluid for cancer cells, where the expression of at least two genes which are selected from the group consisting of (i) manganese superoxide dismutase genes; (ii) thioredoxin reductase genes; and (iii) glutathione peroxidase genes is determined on at least one cell-containing fraction of the body fluid. Claim 2 requires at least one manganese superoxide dismutase gene, at least one thioredoxin reductase gene, and at least one glutathione peroxidase gene to be determined. Claim 11 requires the expression of at least one manganese superoxide dismutase gene and at least one further gene selected from the group consisting of thioredoxin reductase genes and glutathione peroxidase genes to be determined. Claim 3 limits the body fluid to one that is selected from blood, bone marrow, lymph, sputum, lavages, puncture fluids, ascites, mucosal smears, exudates, urine and stool. Claim 4 requires the cell-containing fraction to be obtained from the body fluid with enrichment of cancer cells. Claim 5 limits the cell-containing fraction to one that is obtained from the body fluid with enrichment of cancer cells, and further comprising providing a second cell-containing fraction of body fluid or of a comparable biological sample, determining the expression of the genes in the second cell-containing fraction, and comparing the expression of each gene between the two samples. Claim 6 further limits second cell-containing fraction of claim 5 to one that is derived from the individual whose body fluid is investigated for cancer cells. Claim 7 further limits the method of claim 6 to where it is determined whether expression of the genes in the cell-containing fraction is elevated by comparison with the expression of the genes in the further cell-containing fraction. Claims 12-

17 are directed to the intended uses of the method. Claim 12 indicates that the method is for identifying disseminated cancer cells in the body fluid. Claim 13 further limits the method of claim 12 to where the elevated expression of at least one of the genes indicates the presence of disseminated cancer cells in the body fluid. Claim 14 indicates that the method is for early diagnosis of a tumor. Claim 15 limits the method of claim 14 to where the elevated expression of at least one of the genes indicates the presence of a tumor. Claim 16 indicates that the method is for estimating the risk to develop metastasis or a recurrence. Claim 17 limits the method of claim 16 to where the elevated expression of one of the gene indicates a risk to develop a metastasis or recurrence. The nature of the invention is complex in that carrying out the recited method steps must enable the intended uses of the method, including identifying disseminated cancer cells in a body fluid, providing an early diagnosis of a tumor, and estimating the risk to develop a metastasis or recurrence.

Breadth of the claims: The claims are broad in that the specification defines the term "cancer cell" to mean a cell which exhibits one or more modifications associated with cancer, i.e., dysplasia in the general sense. The term is defined to specifically include precursors of cancer and tumor cells with cancerous or tumorous modifications (e.g., page 4, lines 6-19).

The claims are very broad in that they encompass determining the expression of at lest two genes selected from manganese superoxide dismutase genes, thioredoxin reductase genes, and glutathione peroxidase genes. The specification defines the term "manganese superoxide dismutase (MNSOD)" to mean enzymes which catalyze the decomposition of superoxide free radicals to form hydrogen peroxide, and in particular the enzymes which constitute enzyme class 1.15.1.1 (paragraph bridging pages 14-15). The enzymes of this class are not limited to

manganese-containing superoxide dismutase enzymes (See the entry for 1.15.1.1 from the Enzyme nomenclature databases, accessed from http://us.expasy.org/enzyme). Enzymes of the class 1.15.11 include all superoxide dismutase enzymes, including iron or manganese or copper and zinc superoxide dismutase. Thus, the claims read on determining the expression level of any superoxide dismutase enzyme from any species of organism from which a body fluid may be obtained. The specification defines the term "thioredoxin reductase (TXNRD)" to mean any enzyme that catalyzes the NADPH-dependent reduction of thioredoxin-S_2 to thioredoxin-$(SH)_2$, including the enzymes that constitute enzyme class 1.6.4.5 (page 16, lines 24-28). Thus, the claims read on determining the expression level of any thioredoxin reductase isoform from any species of organism from which a body fluid may be obtained. The specification defines the term "glutathione peroxidase (GPX)" to mean enzymes that catalyze the decomposition of hydrogen peroxide to form water and oxygen, including enzymes that constitute enzyme class 1.11.1.9. Thus, the claims read on determining the expression level of any glutathione peroxidase isoform from any species of organisms from which a body fluid may be obtained. Accordingly, the claims broadly encompass obtaining any body fluid from any species of organism, and determining the expression of at least two of the broadly defined classes of genes selected from the genus of manganese superoxide dismutase genes, the genus of thioredoxin reductase genes, and the genus of glutathione peroxidase genes.

The claims are also broad in that they encompass embodiments without any comparison to a reference sample. Even for those claims that specifically encompass a comparison to a second cell-containing fraction of the body fluid or a comparable biological sample, the claims are very broad. The further cell-containing fraction may be any portion of any type of body

fluid, and the comparable biological sample may be from any body fluid or solid tissue. Accordingly, the claims encompass a large number of different comparisons between the tested cell-containing fraction and a second cell-containing fraction or a comparable biological sample.

The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions using a method for investigating body fluids for cancer cells to permit reliable tumor diagnosis and prognosis (e.g., page 1, lines 5-13).

The specification teaches that the prior art shows that some solid tumors and metastases thereof found in solid tissue have increased expression of MNSOD, including colorectal tumors and hepatic metastases thereof, lung tumors, breast cancer cells, stomach tumors, and gliobalastoma (e.g., page 2, lines 5-22). However, the specification also notes that benign hyperplasias of the breast were often found to be strongly positive for MNSOD expression as compared to neoplastic epithelial cells from invasive carcinomas of the breast (e.g., page 2, lines 22-27). Thus, the specification acknowledges that MNSOD levels are not always higher in dysplastic cells as compared to any cell type. The specification teaches that reduced GPX1 expression was observed in imexon-resistant RPM/8226/I myeloma cells (e.g., page 3, lines 10-12). Thus, gene expression may vary depending upon the sensitivity or resistance of the cancer cell to a cancer therapeutic. Further, the specification teaches that disseminated cancer cells are a tumor entity independent of the primary tumor and therefore are fundamentally different from cells of the primary tumor on the basis of a different genotype and phenotype (e.g., page 3, lines 14-22).

At pages 12-27, the specification provides general guidance directed to measuring expression levels of MNSOD, TXNRD and GPX expression by measuring nucleic acid or protein expression.

At pages 28-30, the specification provides guidance with regard to evaluating the obtained expression levels. The specification teaches that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The specification teaches that the comparison usually is with cells in which no cancer-associated modification is to be expected (non-cancer cells, normal cells) (e.g., page 28, lines 10-14). The specification suggests that if cancer cells in body fluids are being tested, then the comparison will be those normally occurring in this body fluid. For the case of blood, the normal cells are white blood cells which can be obtained for example by density gradient centrifugation (e.g., the buffy coat or the MNC fraction) or can be separated by more specific isolation methods (e.g., CD45-positive lymphocytes) (e.g., page 28, lines 14-22). The specification asserts that these samples can also be used as a comparison for body fluids other than blood (e.g., page 28, lines 22-25). At page 29, lines 33-39, the specification states, "The test principle according to the invention is therefore based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression measured in the comparison cell mixture is decisive." The specification goes on to state, "It will usually be expedient for validation of a particular test system to fix a particular quotient (limit) above which overexpression is present by definition." (See page 30, lines 1-5). Thus, the step of comparing appears to be critical to the claimed invention. Furthermore, the specification notes that the limit

may depend on the cell mixtures used and, in particular, on the obtaining thereof (e.g., page 30, lines 7-8).

With respect to early diagnosis, the specification envisions using sputum/saliva for the early diagnosis of lung tumors; urine for the early diagnosis of prostate and bladder tumors; stool for the early diagnosis of colonic and pancreatic tumors; and blood/bone marrow/lymph for the early diagnosis of all disseminating tumors.

With respect to the prognosis and risk of recurrence, the specification envisions using the method of the invention to classify tumor and estimate risk (e.g., paragraph bridging pages 31-32).

The working examples of the specification are directed to one embodiment that falls within the scope of the instant claims. The examples teach the collection of blood from 9 healthy donors and 47 tumor patients. Breast carcinoma cell line BT474 was used as a reference for MNSOD, TXNRD1, and GPX1 expression. To obtain cancer cell fraction C and comparative fractions A' and B', 10 ml of heparinized blood was centrifuges, and the supernatant plasma was removed. The pelleted cells were resuspended in 12 ml of PBS and subjected to density gradient centrifugation. The mononuclear cell fraction was collected, washed and resuspended in 10 ml of PBS. 1 ml of this cell mixture was removed as a possible reference (comparative fraction A'). The remaining 9 ml of cell mixture was passed via a column through a screen woven from polyester filaments with a 20 μm mesh width, and the flow-through from the screen was collected as a possible reference (cell fraction B'). The column was washed five times with 10 ml of PBS, and the cells trapped on the screen were collected in Trizol® solution (cancer cell fraction C). Comparative fractions A' and B' were further processed by isolating CD45-positive

lymphocytes to obtain comparative fractions A and B. Gene expression was analyzed by TaqMan® analysis of mRNA expression using the following primers and probes: SEQ ID NO: 1 (sense primer for MNSOD), SEQ ID NO: 2 (antisense primer for MNSOD), SEQ ID NO: 3 (probe for MNSOD), SEQ ID NO: 4 (sense primer for TXNRD1), SEQ ID NO: 5 (antisense primer for TXNRD1), SEQ ID NO: 6 (probe for TXNRD1), SEQ ID NO: 7 (sense primer for GPX1), SEQ ID NO: 8 (antisense primer for GPX1), SEQ ID NO: 9 (probe for GPX1), SEQ ID NO: 10 (sense probe for GAPDH), SEQ ID NO: 11 (antisense probe for GAPDH), and SEQ ID NO: 12 (probe for GAPDH). The specification refers to the following accession numbers for MNSOD, TXNRD1, and GPX1: M36693, X91247, and M21304, respectively. GAPDH expression was measured for fractions A or A' and C, and the ratio of the expression of each gene is expressed as a quotient. The specification teaches tat overexpression of the relevant mRNA is present if the ratio of the fraction C quotient to the fraction A quotient is more than a limit which is to be experimentally defined. Further, the specification teaches that cell equivalents are based on a cell standard (e.g., cell line BT474), where cDNA from the cell standard is included in the quantitative analysis in the form of serial dilutions and serves as a reference system. The specification teaches the amounts of MNSOD, TXNRD1 and GPX1 mRNA determined in fraction C as compared to fraction A for healthy donors (e.g., Table 1). The specification teaches that for subsequent assessment of the levels of expression measured in tumor patients, levels were regarded as positive if they exceeded the average level in healthy donors (ratio of level in fraction C as compared to fraction A) plus one standard deviation, as indicated as the limit in Table 1 (e.g., page 45, lines 13-26). MNSOD, TXNRD1 and GPX1 was measured in fractions C and A' obtained from the blood of patients diagnosed with a solid tumor

(e.g., page 46, lines 1-8). Comparing the expression ratios from fractions C and A' to the limits disclosed in Table 1, it was determined that 78/90 (87%) patients were positive for increased MNSOD expression, 60/90 (67%) of patients were positive for increased TXNRD1 expression, and 53/86 (62%) of patients were positive for GPX1. At least one gene was positive in 93% of patients. Thus, detecting all three genes has a sensitivity of 93%, while the sensitivity of the individual detections is 87, 67 and 62%, respectively (e.g., page 47, lines 25-30). Comparison between the healthy donors and some of the tumor patients is shown at pages 50-51. The specification teaches the use of this specific method to detect disseminated cancer cells in patients with solid tumors.

The specification does not teach the stage or grade of the cancers at the time blood was drawn. There is no indication that the cancer cells detected by increased expression of MNSOD, TXNRD1 or GPX1 are not a result of advanced metastatic cancer. The specification does not teach the sensitivity of the assay for early, non-metastatic cancer.

With respect to estimating the risk to develop metastasis or recurrence, the specification teaches the comparison between tumor patients with out recurrence and those with recurrence in relation to MNSOD, TXNRD1, and GPX1 expression as discussed above (e.g., pages 52-53). While some statistical differences were observed, the percentages disclosed in Table 7a for carcinoma of the breast and Table 7b for tumor patients, indicates that may not be able to use the expression levels of MNSOD, TXNRD1 and/or GPX1 to reliably classify a single test individual as at risk or not at risk of recurrence.

The specification discloses probes that could be used for microarray analysis of MNSOD, GPX2, GPX3, and TXNRD1 (e.g., page 55). The specification asserts that overexpression of

MNSOD and GPX2 is clearly evident upon hybridization of mRNA total amplification from a tumor cell fraction C as compared to cell fraction A' (e.g., page 57 and Figure 1).

The specification does not teach the expression of MNSOD, TXNRD or GPX in body fluids such as bone marrow, lymph, sputum, lavages, puncture fluids, ascites, mucosal smears, exudates, urine or stool. The specification does not contain working examples directed to the early diagnosis of a tumor or risk of developing a metastasis.

Predictability and state of the art: The art teaches that gene expression analysis is commonly used for three different purposes: (1) as a screening tool to identify individual genes of interest that might contribute to an important biological function, (2) to obtain insight into an important biological function, and (3) as a classification tool to sort cases into clinically important categories (Pusztai and Hess, Annals of Oncology, Vol. 15, pages 1731-1737, 2004; e.g., paragraph bridging pages 1732-1733). Pusztai and Hess teach that validation of gene expression important to biological function may be validated by using different methods, such as RT-PCR, whereas the most appropriate validation for using gene expression analysis as a classification tool is testing the predictor on independent sets of cases (e.g., page 1733, left column, 1st full paragraph). In the instant case the specification does not teach that the expression levels can be used to reliably categorize an individual. For example, the specification does not teach the classification of individuals as at risk or not at risk to develop a metastasis, or at risk to develop a recurrence.

Further, Shalon et al (US 2001/0051344 A1, Dec 13, 2001) teach that due to variations in genetic make-up of unrelated individuals in a heterogeneous society, differences in the expression of a gene between any two individuals may or may not be significant (e.g., paragraph

[0155]). Shalon et al further teach that the larger the number of individuals tested, the more significant the remaining differences in gene expression become and samples from at least 5 and preferably 20-50 different test individuals are assayed to obtain statistically meaningful data showing a statistical elevation or reduction in report levels when compared to control levels (e.g., paragraph [0156]). Pusztai and Hess teach that larger samples sizes may be needed to validate classification tests, and the number of samples will vary depending upon the acceptable error rates, level of inter-patient variability, the size of the difference in mean expression values, and the prevalence of the phenotype among the group being tested (e.g., page 1734, paragraph bridging columns; Table 1).

Genetic tests are heterogeneous in nature and the exact characteristics of a particular genetic test to be evaluated must be tightly defined (Kroese et al (Genetics in Medicine, Vol. 6, pages. 475-480, 2004). Kroese et al teach that genetic test is shorthand to describe a test to detect a particular genetic variant for a particular disease in a particular population and for a particular purpose and that it should not be assumed that once the characteristics of a genetic test are evaluated for one of these reasons that the evaluation will hold or be useful for other purposes and all measures of the test performance should be presented with their 95% confidence intervals (e.g., page 477, 1st column, 1st and 2nd full paragraph). Kroese et al teach that the limitations of our genetic knowledge and technical abilities means that for the moment there are likely to be gaps in the information needed to complete a thorough evaluation of many genetic tests (e.g., page 479, 2nd column, last paragraph).

The prior art reveals that differences in gene expression observed between two groups are do not necessarily provide markers that can be used to reliably classify a subject. Golub et al

(Science, Bol. 286, pages 531-537, October 1999) teach the use of a two-step procedure to test the validity of gene expression levels as predictors: step 1 involves cross-validation of the predictors on the initial data set, where one withholds a samples, builds a predictor based only on the remaining samples and predicts the class of the withheld sample; step 2 involves the repetition of assessing the clinical accuracy of the predictor set on an independent set of samples (e.g., page 532, right column). Although Golub et al could detect gene expression differences between chemotherapy responders and non-responders, those differences could not be use to predictably classify individuals (e.g., page 533, paragraph bridging left and middle columns). Accordingly, the art demonstrates the unpredictable nature of extrapolating gene expression differences to a method of class prediction.

The art teaches that different isoforms of MNSOD, TXNRD1 and GPX1 are expressed (See the Entrez Gene entries for SOD2, TXNRD1, and GPX1 downloaded from http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene on 5/5/2008). These genes correspond to the genes detected by the primers recited in the present specification. The specification does not specifically teach the increased expression of each isoform in circulating cancer cells. Thus, it would be unpredictable to detect any isoform of these genes for the use of the claimed method. Moreover, Seven et al (Clinical Biochemistry, Vol. 32, No. 5, pages 369-373, 1999) teach that the constitutive levels and the inducibility of antioxidant enzymes including superoxide dismutase and glutathione peroxidase vary for different tissues, and the expression of these enzymes may vary according to the type of cancer or tissue studies, resulting in controversy in the literature (e.g., page 372, left column, last two paragraphs). Seven et al did not find an

increased amount of CuZn SOD or glutathione peroxidase in the red blood cell fraction of laryngeal cancer patients (e.g., page 372, paragraph bridging columns; Table 1).

Amount of experimentation necessary: The quantity of experimentation necessary to carry out the full scope of the invention is large. One would be required to conduct a large number of experiments to test the expression of many manganese superoxide dismutase genes, thioredoxin reductase genes, and glutathione peroxidase genes, in combinations of two, from cell-containing fractions of body fluid, including blood, bone marrow, lymph, sputum, lavages, puncture fluids, ascites, mucosal smears, exudates, urine and stool in a number of different species of organisms. Given the variable expression of the enzymes based upon tumor or cell type and the expression of multiple different isoforms, it would be unpredictable to extrapolate the results of the present specification to the use of any manganese superoxide dismutase gene, any thioredoxin reductase gene, and/or any glutathione peroxidase gene. As discussed in the present specification, the limit used to determine whether a gene is overexpressed must be experimentally determined for each particular comparison. This comparison will be specific for the organism, body fluid, cells collected from the body fluid, gene whose expression is determined, isoform whose expression is determined, whether mRNA or protein expression is measured, the specific method used to measure the mRNA or protein (e.g., RT-PCR, microarray, or ELISA), whether enrichment is used, and the type of control sample. A large amount of unpredictable experimentation would be required to use the full scope of the claimed method to detect the presence of disseminated cancer cells, provide early diagnosis of a tumor, estimate the risk to develop a metastasis, or estimate the risk to develop a recurrence.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1-7 and 11-17 are not considered to be fully enabled by the instant specification.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3 and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Seven et al (Clinical Biochemistry, Vol. 32, No. 5, pages 369-373, 1999; see the entire reference).

The claims are drawn to the determination of expression of at least two genes which are selected from the group consisting of manganese superoxide dismutase genes, thioredoxin reductase genes, and glutathione peroxidase genes. The specification defines the term "manganese superoxide dismutase" to encompass any superoxide dismutase gene of the class 1.15.1.1 (paragraph bridging pages 14-15).

Regarding claims 1 and 11, Seven et al teach the determining the expression of CuZn superoxide dismutase (CuZn SOD), and glutathione peroxidase (GSH Px) in the erythrocyte-containing cell fraction of blood (e.g., page 370, left column; Table 1). The CuZn SOD taught by Seven et al falls within the scope of the claims as a manganese superoxide dismutase gene based upon the explicit definition provided in the instant specification.

Regarding claim 3, Seven et al teach the method where the erythrocyte-containing cell fraction is obtained from blood (e.g., page 370, left column).

Claims 1, 3 and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Kizaki et al (Blood, Vol. 82, No. 4, pages 1142-1150, August 1993; see the entire reference).

Regarding claims 1 and 11, Kizaki et al teach determining the expression of manganese superoxide dismutase (Mn-SOD), and glutathione peroxidase (GSX-PX) on the peripheral blood lymphocyte-containing fraction of blood (e.g., Abstract; page 1143, left column, 1st full paragraph; page 1143, Northern blot analysis; Figure 3C).

Regarding claim 3, Kizaki et al teach the method where the peripheral blood lymphocytes are obtained from blood (e.g., page 1143, left column, 1st full paragraph).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications

may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

/Celine X Qian Ph.D./
Primary Examiner, Art Unit 1636